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RESEARCH ARTICLE

MORPHOTYPES OF CANDIDA ISOLATES FROM ORAL LESIONS ON SABOURAUD-TRIPHENYLTETRAZOLIUM AGAR

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ABSTRACT

Introduction: Candidiasis is found to be associated with a number of oral lesions, either as a cause or as an agent of supra added infection. However, the correlation between various candida subspecies and oral lesions still appears to be unclear. Various typing methods have been used previously for differentiation of candida subspecies, but have been found to be relatively complicated. In the present study, we present a simple and easy typing method using Sabouraud-Triphenyltetrazolium Agar (STTZ) as a tool for differentiation and morphotyping of candida subspecies differentiation associated with oral lesions.

Methodology: A total of 101 candida strains were isolated from patients with oral lesions. The STTZ agar was prepared as per the method described by Quindós *et al* (1992). After incubation of cultures at 37°C for 6 days, a three-letter code was given to each strain, according to its colonial morphology. Association between morphotypes and oral lesions as well as morphotypes and candida species was evaluated.

Results: *Candida albicans* was found to be the most predominant species amongst the 101 oral lesions studied, representing a total of 8 morphotypes. SP1N type (smooth, pale pink, without mycelial halo) was the predominant morphotype for *Candida albicans*. Morphotyping of other candida species could also be done effectively using this method.

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INTRODUCTION

Candidiasis is a common opportunistic fungal infection of the oral cavity. (Parihar, 2011) Many of the oral lesions have been reported to be associated with *Candida albicans* and certain other species such as *C. glabrata, C. tropicalis, C. krusei* and *C.parapsilosis*. (Meurman *et al*., 2007) It has also been speculated that there is a correlation between the candida types and the virulence of isolates of Candida. (Hunter *et al*., 1989; Merz, 1990) Different typing methods have been described previously for differentiation of candida sub species including biotyping (Odds and Abbott, 1980), enzyme typing (Roman *et al*., 1983), morphotyping (Phongpaichit *et al*., 1987), serotyping (Hasenclever *et al*., 1983), resistotyping (McCreight *et al*., 1985), killer typing (Polonelli *et al*., 1983), protein typing (Howell and Noble, 1990) and karyotyping. (Merz *et al*., 1988) Attempts have also been made to conduct the morphotyping of various strains of candida associated with oral cavity but most of these methods are relatively complicated. (Hunter *et al*., 1989 The present study explains a simple and easy morphotyping method by using Sabouraud-Triphenyltetrazolium Agar (STTZ) as a tool for differentiation of candida subspecies associated with oral lesions.

MATERIALS AND METHODS

Approval for the study was obtained from the Institutional Research Board and Ethics Committee. Patients with oral lesions reporting to the Department of Oral Medicine & Radiology were included in the study after obtaining their informed consent. Some of the lesions included in the study have been shown in Fig.1. A pre-designed proforma was used to record their details, description and diagnosis of the oral

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lesions. Sample collection was done by passing a sterile cotton swab several times across the affected area with the lesions and for some cases, like denture wearers, swabbing was done from oral mucosa, tongue (including dorsum of tongue) and soft palate. For keratotic lesions like leukoplakia, scrappings were taken with sterile spatula. The specimens were transported to the laboratory and the swabs were inoculated on Sabouraud Dextrose Agar (SDA) and incubated at 37^0 C for 24 hrs. All the isolates were identified by standard laboratory techniques. (Larone, 2002) The Sabouraud-triphenyltetrazolium (STTZ) agar containing 2, 3, 5-triphenyltetrazolium chloride (0.1 g) (Sigma Chemical Co., Louis, Mo.), peptone (10 g), glucose (20 g), agar (20 g), and distilled water (1 liter), was prepared as per the method published by Quindós *et al*. (1992) Fresh 24 hr growth of the fungus on Sabouraud Dextrose Agar (Himedia) was used for typing. Briefly to prepare each inoculum, three to four colonies were suspended in 1 ml of distilled water and the turbidity was adjusted to 5 MacFarland standard. STTZ plates were inoculated with 10 ul from the cell suspension by using a micropipette and allowing the inoculum to drop and dry on the agar surface. The plates were then incubated at 37°C for 6 days. After incubation the colonies were examined. (Fig 2) A three-letter code was given to each strain, based on (Guillermo Quindos *et al*., 1992):

1. Texture whether smooth (S) or rough (R).
2. Color: (i) Pink (P1 = Pale pink = Pantone 176C, P2 =Dark pink = Pantone 1785C), (ii) Violet (V1 = Pale violet = Pantone 251C, V2 = Dark violet = Pantone 262C)(Pantone Inc.),(iii) Orange (O),(iv) White (W)
3. Presence of mycelial halo (hyphae or pseudohyphae): Y= yes and N = no.

The code NG was used if no growth was observed. For example if isolate was smooth, pale pink, without mycelial halo, then the morphotype was SP1N. Pantone is a registered trademark for color specification that is oriented for facilitation and standardization of color for publishing and graphic design work.

Data was analyzed by using SPSS (version 22.0). Association between morphotypes and oral lesions as well as morphotypes and candida species was evaluated by using Chi-Square Tests.

RESULTS

A total of 101 candida strains were isolated from equal number of patients with oral lesions**.** *Candida albicans* was found to be the most predominant species amongst all the isolates. In all 8 morphotypes were found. *Candida albicans* showed 7 different morphotypes and SP1N type (smooth, pale pink, without mycelial halo) was the most predominant morphotype for this species. While *C.krusei* showed 2 types of morphotypes (SP1N&SV2N), *C.guilliermondii* (SP2N,SV2N, RV2N), *C.tropicalis* (SP1N,RP1N,RP1Y), *C.glabarata* (SP2N, SV2N, RP1Y) showed 3 types of morphotypes (Graph 1). SP1N type of morphotype associated with different candida species, was maximally seen in Pseudo-membranous candidiasis and leukoplakia ($p<0.01$). Leukoplakia was associated with 5 types of morphotypes and SP1N type was predominantly recovered from 75% of the cases. Pseudomembranous candidiasis showed 6 types of morphotypes with 50% of isolates classified as SP1N type of morphotype. Angular chelitis showed only SP1N type of morphotypes. Clinically diagnosed malignant lesions showed all types of morphotypes with the exception of RP2N and as many as 42% of these lesions were again SP1N morphotype. (Graph 2)

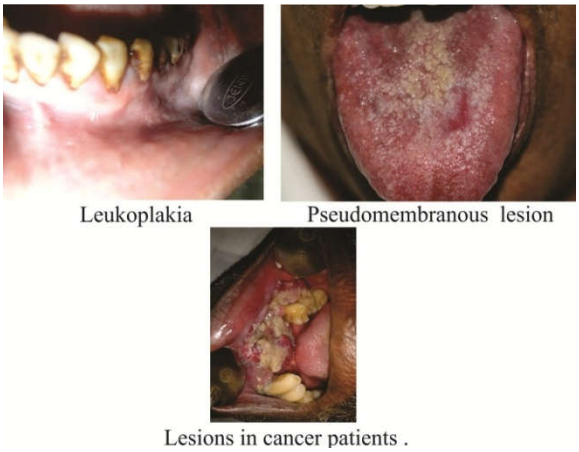


Figure 1. Oral lesions

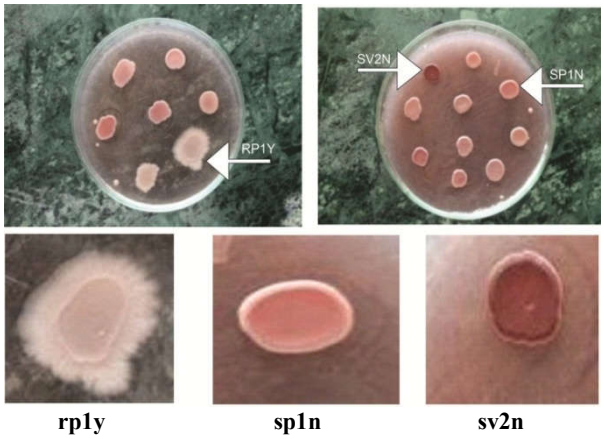
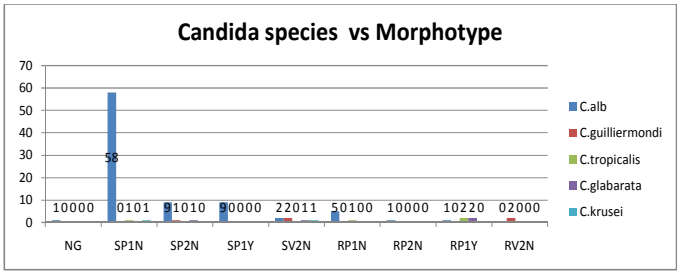
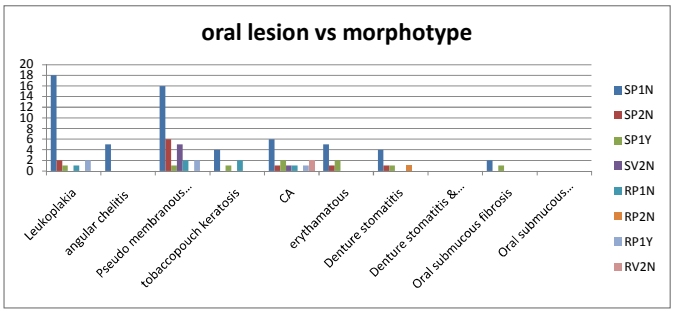


Figure 2. STTZ Agar showing different morphotypes like RP1Y (Rough, pale pink with mycelial halo, SP1N (Smooth pale pink without mycelial halo), SV2N(Smooth, dark violet without mycelia halo)



Graph 1. Candida species vs morphotypes of Candida



Graph 2. Different oral lesions against different morphotypes of Candida

DISCUSSION

In this study, a simple method was assessed for morphotyping of candida isolates from various oral lesions. To the best of our knowledge this is probably the first study in india in oral lesion to evaluate Sabouraud-Triphenyl Tetrazolium Agar (STTZ) as a tool for morphotyping of candida subspecies differentiation associated with oral lesions. Some other methods have been attempted by various researchers in the past for morphotyping of Candida. In 1987 Phongpaichit's morphotyping method (Phongpaichit *et al*., 1987), numerical codes were assigned primarily on the basis of the nature and extent of marginal fringing and the surface topography of the streak colony. This system allows ready differentiation to be made of morphotypes, requires no specialized equipment or expertise. Though this method provides a simple and reproducible means for epidemiological studies of candida and candidiasis, it is not useful for other Candida species. Our STTZ agar method can be used for different candida species. Another morphotyping method described by Hunter *et al*. (1989) has been shown to possess a good discriminatory capacity. (Hunter *et al*., 1989) The combination of simplicity with good discrimination made this morphotyping an ideal typing method for first-line use in old days.However, Hunter method is complicated, doesn't show any color and usually cannot been applied to other Candida species. Earlier using the same STTZ agar method, Quindos et al. (1992) have reported 16 morphotypes from 562 unrelated candida strains of which predominant morphotype was SP1N. Similar results were observed in our study. SP1N morphotype was more common in invasive blood samples as well as in oral samples. These results were also similar to our study. None of T.glabarata (now *C. glabararta*) had grown on STTZ agar in the former study, while in our study *Candida glabarata* showed 3 types of morphotypes (SP2N, SV2N, RP1Y). (Guillermo Quindos *et al*., 1992)

S. Gamarra in 2015 also used STTZ agar method for differentiation of *C.dublinensis* and *C.albican* isolated from various samples such as blood and urine. (Gamarra *et al*., 2015) It can be the another use of STTZ agar method. Candida dublinensis was not isolated from any samples in our study. Although we were not able to make a direct comparison between the other morphotype methods and STTZ agar method, it was evident from this study that it is a relatively simple and easy method for detailed morphotyping of candida isolated and can be applied for morphotyping of a range of candida species. The simplicity could make this method useful epidemiological tool for the typing of Candida species in different oral lesions. The limitation of this study is that the number of isolates in each of the oral lesions is small but the fact that RP2N morphotype was not found in any of the malignant lesions clearly shows that the scheme has great exploratory potential for studying the possible correlation between different candida morphotypes and nature of the oral lesions. At present there is some clustering of SPIN morphotypes in certain conditions but in Angular chelitis this was an exclusive morphotype. With larger number of study subjects this issue may be resolved and the method may prove to be a stepping stone in the unchartered territory of clear association between candida species and oral lesions.

Conclusion

Morphotyping of Candida isolates from oral lesions on Sabouraud- Triphenyltetrazolium (STTZ) Agar proved to be a simple and easy typing method for Candida subspecies differentiation. Compared with other systems for strain differentiation, the STTZ agar morphotyping system also has the benefit of morphotyping of variety of candida species other than *C. albicans*. Considering this, it can easily be applied to study the correlation between candida subspecies and oral lesions.

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